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THE PRESERVATION OF PLANKTON GRAB SAMPLES

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Cincinnati, Ohio

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ABSTRACT

The bactericidal and algicidal properties of formalin, mercuric chloride, and Merthiolate were tested using grab river-water samples and cultures of Scenedesmus bijuga and Cyclotella meneghiniana. Formalin was bactericidal in plankton grab samples at a concentration of 1.0%. Cyclotella failed to grow in 0.01% formalin (the lowest concentration of formalin tested). The growth of Scenedesmus was delayed briefly in 0.01% formalin, and completely arrested at a concentration of 0.1% or more of formalin.

Mercuric chloride rendered grab samples sterile at concentrations of 10-20 mg of mercury per liter. The growth of Cyclotella was not affected by 0.5 mg or less of mercury per liter, but was retarded at higher concentrations. This alga did not grow at mercury concentrations of 10 mg or more per liter. The growth of Scenedesmus was briefly delayed by as little as 0.25 mg of mercury per liter, but it grew slowly (after a lengthy lag phase) at mercury concentrations as high as 10 mg per liter.

The Merthiolate-sodium borate-iodine preservative used in Water Pollution Surveillance System samples was algistatic but not

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INTRODUCTION

The preservation of plankton grab samples has changed little in recent years despite the availability of a great variety of antibiotic substances. The preservative most widely used today is formalin. Long experience has shown that this substance prevents biodegradation of the phyto- and zooplankton in samples (Welch, 1950), but it falls short of being an ideal preservative in several important respects: (a) preserved samples emit irritating vapors, (b) frequent contact with solutions containing formalin causes the skin to crack and roughen, and (c) algal pigments deteriorate rapidly, making identifications more difficult as the samples age.

Mercurials have long been used as preservatives and disinfectants by microbiologists, but have not been generally employed for the preservation of plankton samples. Among the few reports of the use of mercurials for this purpose are those by Williams (1962) who adopted Merthiolate in 1960 as a preservative for plankton grab samples collected by the Water Pollution Surveillance System, and Williamson (1963), who employed ethyl mercuric chloride and mercuric iodine to preserve marine net plankton collected with an automatic sampler.

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The ideal plankton preservative would be: (a) non-volatile and non-irritating to human skin, eyes, and respiratory system, (b) chemically stable in solution and have a long shelf life, (c) inexpensive, (d) toxic to all microorganisms at very low concentrations, (e) a good preservative of chlorophyll and other cell pigments, and (f) unaffected by large amounts of dissolved and suspended organic and inorganic matter commonly occurring in water samples.

The purpose of this report is to compare the bactericidal and algicidal properties of three preservatives - formalin, mercuric chloride, and Merthiolate, and to stimulate renewed interest in the search for better methods of stabilizing plankton in grab samples.

The author gratefully acknowledges the laboratory assistance of Gretchen Cooper, Donald Moore, and Donald Stevens.

METHODS

Bacteriological Tests

Grab water samples were collected from the Ohio or Little Miami Rivers in the Cincinnati area, and treated with various levels of preservatives. Aliquots of raw water were plated for total counts immediately after the samples were collected, and again 24 hours to 10 days after treatment. Total plate-count agar was used for all tests, and plates were incubated three days at ambient laboratory temperatures before counting. The bacteria plates were prepared and counted by the microbiology unit of the Water Pollution Surveillance System.

Algal Bioassays

Algal bioassays were conducted with pure cultures of Scenedesmus bijuga (Turp.) Lagerh. and Cyclotella meneghiniana Kutz.

Scenedesmus bijuga is a common planktonic coccoid green alga which in culture occurs as a mixture of single cells, doublets, and colonies of four and eight cells (Figure 1a). The cells measure 4-8μ in diameter and 7-16μ long. In reproduction, each cell divides to form 2, 4, or 8 cells which may or may not remain attached to one another as they mature. The strain used in this study was obtained from the culture collection of algae maintained by Dr. Richard Starr, Department of Botany, University of Indiana, Bloomington.

a b

Figure 1. Algae used for bioassay.
(a) Scenedesmus bijuga, (b) Cyclotella meneghiniana

Cyclotella meneghiniana is one of the most abundant planktonic centric diatoms. The cells are cylindrical, measuring 4-12μ in diameter and 7-15μ long (Figure 1b), and separate soon after dividing. The strain used in this study was isolated by the author from the Little Miami River, Cincinnati, in June 1965.

Stock cultures of both algae were maintained in modified Chu Medium #10 (Chu, 1942) at 75°F, with c.a. 250 f.c. of daylight fluorescent illumination and a 12:12 diurnal photoperiod.

Bioassays were carried out in modified Chu Medium #10, or filter-sterilized Ohio or Little Miami River water. Experimental cultures contained approximately 50,000 cells/ml in order to duplicate the phytoplankton population density of samples collected at our more productive stations. The conditions of light and temperature were those described above. Growth was determined periodically by measuring the optical density of the cultures at 600 mu (light path 17mm) with a Bausch and Lomb Spectronic 20 Spectrophotometer (Rodhe, 1948, Gross and Kaczy, 1946). Optical density measurements were generally taken every other day for the first two weeks, and then weekly, bi-weekly, and monthly as the cultures aged.

FORMALIN

Many field biologists confuse the terms "formalin" and "formaldehyde". Formaldehyde is prepared commercially by passing a mixture of methanol vapor and air over a catalyst at high temperature (Noller, 1957). The resulting formaldehyde and the excess methanol are absorbed in water, and further concentrated by distillation. This solution, called FORMALIN,

when marketed, contains 37% formaldehyde and 5-10% methanol. Plankton samples are usually preserved by adding 5 ml of formalin for each 95 ml of sample volume (Welch, 1948).

Formaldehyde is very reactive chemically, and causes stasis and death of microorganisms by denaturing proteins (enzymes) and reacting with a wide variety of cellular constituents. Occasionally, cellular changes caused by formaldehyde are so extensive that identification is rendered impossible. Lackey (1939) compared the condition of 234 species of planktonic protozoa (including many green flagellates) in raw and formalin-preserved samples, and found that formalin often caused distortions in shape, loss of flagella, shrinkage, and many other undesirable effects. Thirty-three percent of the preserved organisms could not be identified to species, and a few disintegrated completely. He recommended the use of a 4-5% solution of formalin for preservation, however, stating that lower concentrations killed too slowly and allowed time for greater cell distortion and rupture.

Bacteriological Tests

A grab sample from the Ohio River was plated for an initial bacteria count, and aliquots were treated with reagent and technical grade formalin at the following concentrations: 0.001%, 0.01%, 0.1% and 1.0%. The treated and untreated materials were replated after standing 24 hours on the shelf. Total bacteria counts were unaffected by 0.001% formalin, but were reduced 90% by 0.01% formalin, and 99.99% by 0.1% formalin. Apparent sterility was achieved with 1.0% formalin (Figure 2).

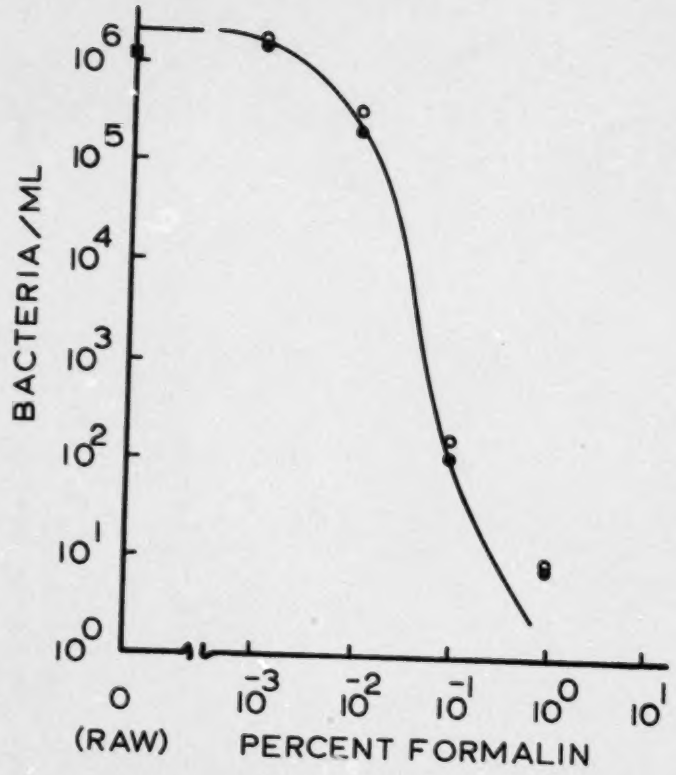


Fig. 2. The effect of reagent and technical grade formalin on the total bacteria counts in a plankton grab sample from the Ohio River (0 = reagent grade; ● = technical grade).

Two replicate tubes of brain-heart infusion broth were inoculated with material from each treatment to determine whether the effect was bacteriostatic or bactericidal. In this test, no growth was observed in tubes inoculated with material which had been treated with 1.0% formalin (0.37% formaldehyde), indicating a cidal effect (Table 1). Growth did occur, however, in tubes inoculated with samples preserved at lower formalin concentrations. There was no difference between the effects of the reagent and technical grade formalin.

Table 1. Brain-Heart Infusion Tube Test for Stasis

Formalin Concentration	Reagent Grade Formalin	Technical Grade Formalin
1.0%	-,-	-,-
0.1%	-,+	+,+
0.01%	+,+	+,+
0.001%	+,+	+,+

(+) growth
(-) no growth

Algal Bioassays

Cultures of Scenedesmus and Cyclotella were treated with formalin at the following concentrations: 0.01%, 0.1%, 0.5%, 1.0%, and 2.0%. The Scenedesmus culture in 0.01% formalin resumed normal growth after a lag period of two weeks (Figure 3), but no growth of Scenedesmus

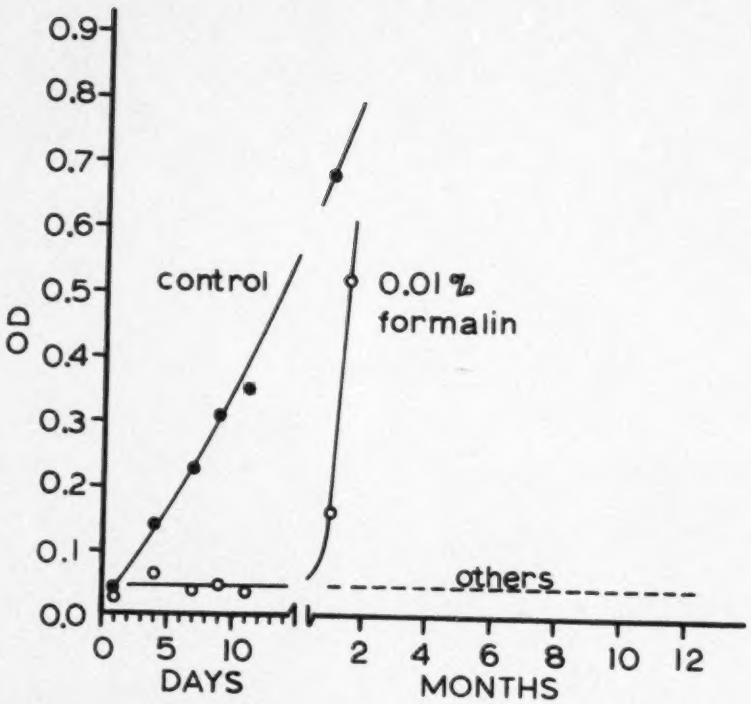


Fig. 3. The effect of formalin on the growth of Scenedesmus ("others" include 0.1%, 0.5%, 1.0%, and 2.0% formalin).

was observed at higher concentrations of formalin. Cyclotella failed to grow at even the lowest formalin concentration (0.01%). The formalin-treated cultures were examined monthly for a period of one year and showed no further signs of growth.

These data indicate that a concentration of 5% formalin provides a very large algistatic and bacteristatic safety margin. A concentration of 2% formalin would undoubtedly be great enough to ensure preservation of the plankton and at the same time substantially reduce the amount of irritating vapors which normally plague those who work with the samples. These remarks do not apply, however, to the use of formalin in biological samples concentrated by centrifugation, netting or sieving.

MERCURIC CHLORIDE

The use of $HgCl_2$ as a disinfectant and preservative was popularized by Koch, who demonstrated the powerful germicidal properties of this substance in 1881. Geppert (1889) and others later found that the effects of inorganic mercurials were primarily bacteriostatic, rather than bactericidal, but this distinction is of negligible importance in plankton preservation. Mercuric chloride has been employed as a skin disinfectant, leather fungicide, insecticide for cabbage maggot, to sterilize medical instruments, and to treat seed potatoes, timbers, and paper products (Brewer, 1954). Although it is generally used at a concentration of 1 gm per liter (at pH 5.9), concentrations as low as 8 mg per liter were found to kill bacteria in as little as 10 minutes (Birkhaug, 1933).

It is generally agreed that mercurials arrest the growth of microorganisms by complexing the sulfhydryl groups of enzymes and other essential metabolities (Brewer, 1954). The effects of mercurials can be prevented, or even reversed, by the addition of glutathione, cysteine, and other sulfhydryl-containing compounds (Fildes, 1940; Barron and Singer, 1945). Brewer (1948) was able to demonstrate this in vivo by reactivating mercurochrome-treated Streptococcus after it had been injected into mice. This was done by the intramuscular administration of 2, 3-dimercaptopropanol (British Anti-Lewisite). The BAL neutralized the mercurochrome in the Streptococcus, freeing it from the bacteriostatic restraint. All mice (20) receiving the BAL treatment within two hours after infection died, whereas only two of 20 infected controls died.

It may be assumed that the temporary delays in algal growth observed in our cultures were due to the inactivation of a sufficiently large portion of the cellular enzymatic systems to prevent cell division, but that metabolism continued at a reduced rate until the affected molecules were replaced, or the mercury became bound to other less essential organic substrates.

The chief disadvantages in the use of mercuric chloride are its corrosiveness and high human toxicity. Furthermore, at low concentrations the amount of $HgCl_2$ required for bactericidal or bacteriostatic effects is proportional to the total amount of organic matter in the sample.

Bacteriological Tests

Two grab samples from the Ohio River and one from the Little Miami River (all collected on different dates) were treated with mercuric chloride to give concentrations of mercury (Hg) ranging from 0.01 to 100 mg per liter. Concentrations of 1 mg Hg or less per liter had no effect on bacteria counts (Figure 4). Counts were sharply reduced, however, in the range of 2.5 to 5.0 mg Hg per liter, and apparent sterility was achieved in the range of 10 mg to 20 mg Hg per liter. The effects of mercury on bacterial activity observed in these studies were not as great as those reported by the American Society for Testing and Materials Subcommittee on Toxicity of Industrial Wastes (Ingols, 1954), which obtained a 90% reduction in the BOD of diluted sewage treated with 1.1 mg Hg (as $HgCl_2$) per liter, and complete bacteriostatis at 1.5 mg Hg per liter. Since the concentration of mercury required to sterilize a plankton sample would depend upon the pH and amount of dissolved and suspended organic matter (Miller and Rose, 1939), some variation in response would be expected from sample to sample. The grab samples used in these tests were collected when the river stage and water turbidity were high. Because sterility was achieved with 20 mg Hg per liter under these conditions, it is reasonable to assume that this concentration of mercury (as $HgCl_2$) would be sufficient for routine sample preservation.

Algal Bioassays

Two series of cultures of Cyclotella and Scenedesmus were treated with mercuric chloride. The first series was prepared with the following concentrations of mercury: 0.001, 0.01, 0.1, 1.0, and 10.0 mg per liter.

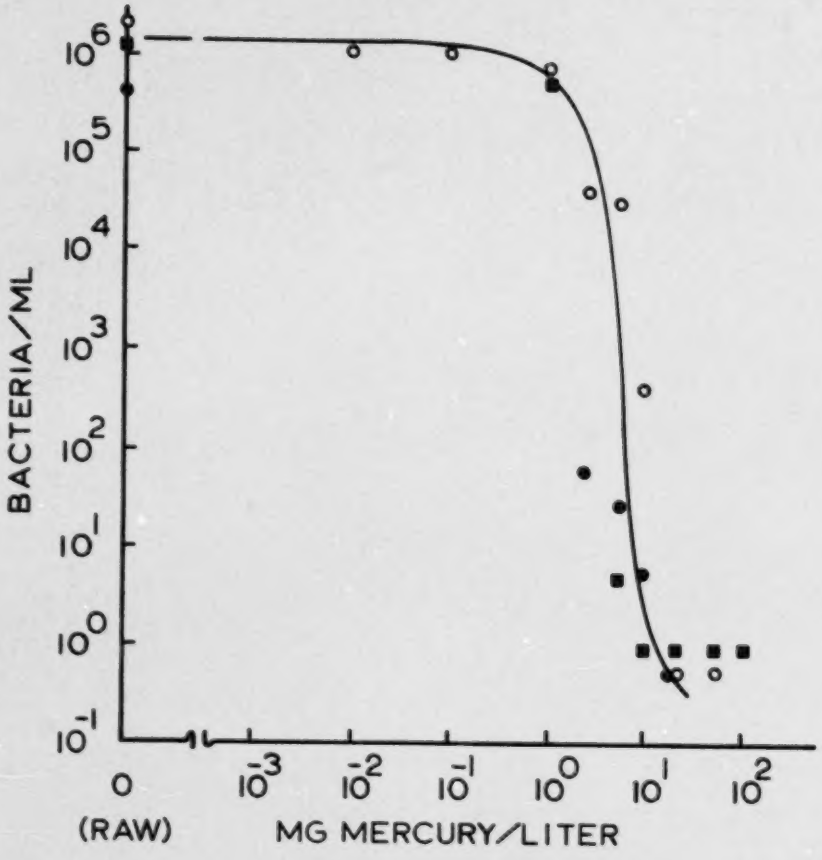


Fig. 4. The effect of mercuric chloride on the total bacteria counts in plankton grab samples (● = Ohio River, Sample A; ■ = Ohio River, Sample B; O = Little Miami River Sample).

The growth of Cyclotella was not measurably affected at concentrations of mercury less than 1.0 mg per liter (Figure 5). Growth was briefly retarded at 1.0 mg Hg per liter, but within 10 days this culture had approximately the same optical density as the control. The culture containing 10.0 mg Hg per liter failed to resume growth over a period of 14 months.

Scenedesmus was more sensitive to mercuric chloride than Cyclotella. The growth of Scenedesmus was delayed more than two weeks by 1.0 Hg per liter (Figure 6).

When no growth was observed in two weeks in cultures of either species containing 10.0 mg Hg per liter, a second series of treatments was prepared with both species of algae. Cyclotella cultures were treated with 0.1, 0.5, 1.0, 2.5, and 5.0 mg Hg per liter, and Scenedesmus was treated with 0.1, 0.25, 0.50, 0.75, and 1.0 mg Hg per liter. In this series, the growth of Cyclotella was not inhibited by 0.1 or 0.5 mg Hg per liter, but was again briefly delayed at 1.0 mg Hg per liter. Growth was delayed one week at 2.5 mg Hg, and six weeks at 5.0 mg Hg per liter (Figure 7). After one year, cells in the earlier culture with 10.0 mg Hg per liter appeared dead, but their viability was not tested by subculturing.

The growth of Scenedesmus was delayed a few days at 0.25 mg, approximately one week at 0.50 mg and two weeks at 0.75 mg and 1.0 mg Hg per liter (Figure 8). The culture containing 10.0 mg Hg per liter resumed a slow growth after three months. This culture still

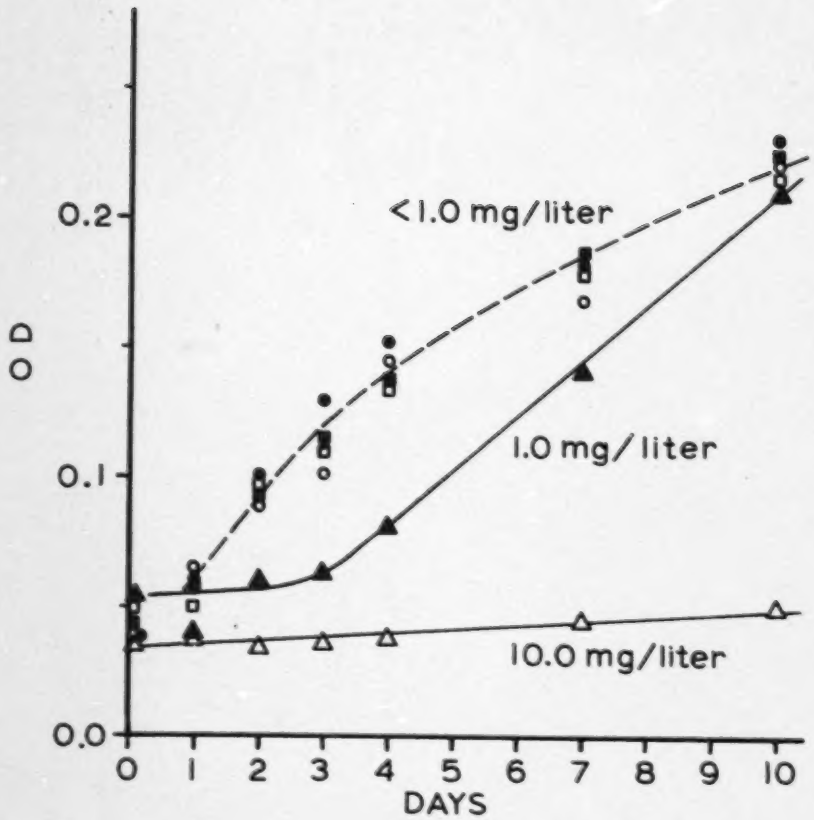


Fig. 5. The effect of mercuric chloride (as mg Hg per liter) on the growth of Cyclotella (● = control; 0 = 0.001 mg; ■ = 0.01 mg; □ = 0.1 mg).

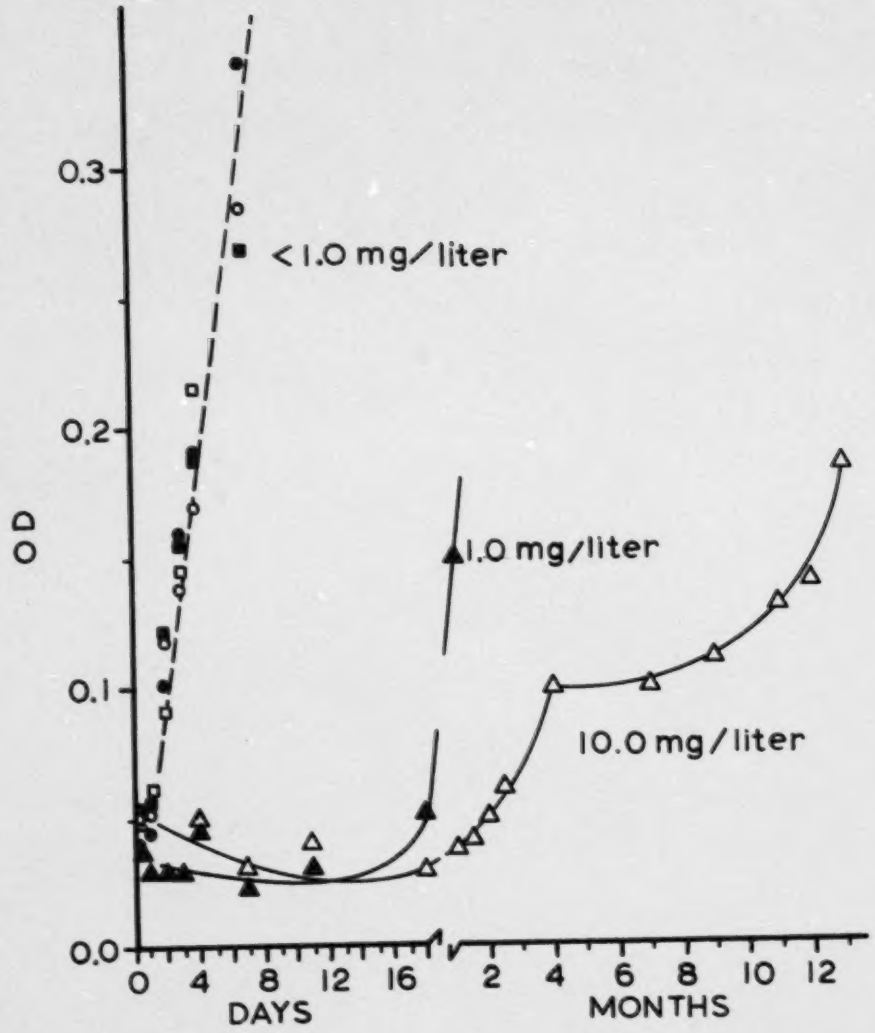


Fig. 6. The effect of mercuric chloride (as mg Hg per liter) on the growth of Scenedesmus (● = control; - = 0.001 mg; ■ = 0.01 mg; □ = 0.1 mg).

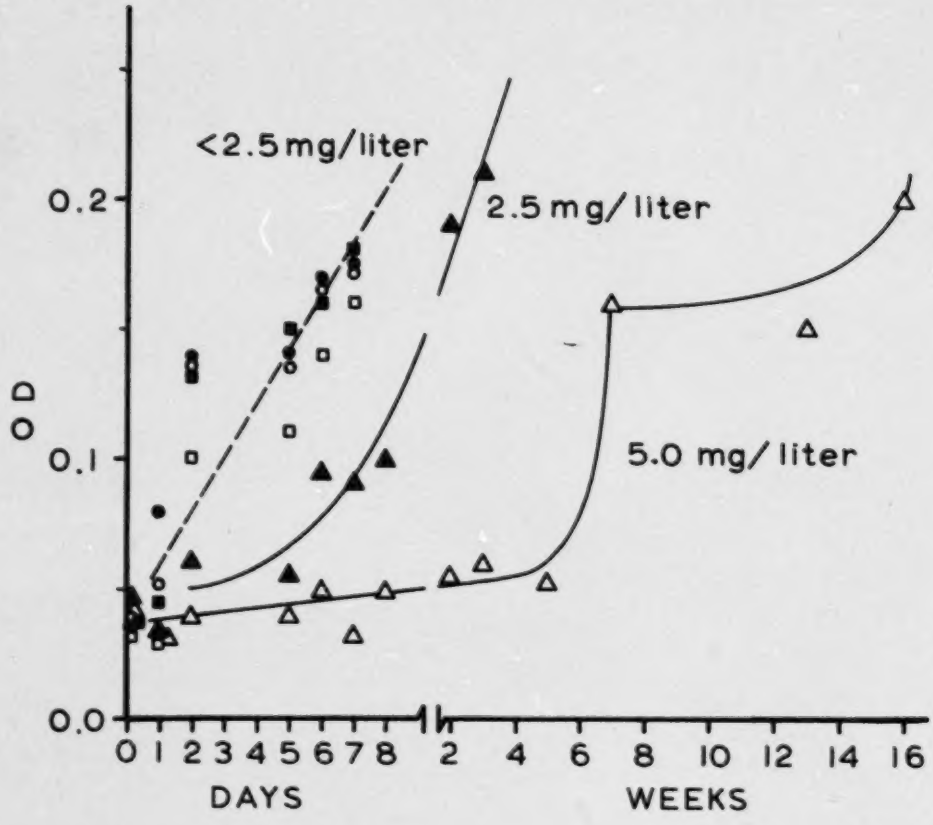


Fig. 7. The effect of mercuric chloride (as mg Hg per liter) on the growth of Cyclotella (● = control; 0 = 0.1 mg; ■ = 0.5 mg; □ = 1.0 mg).

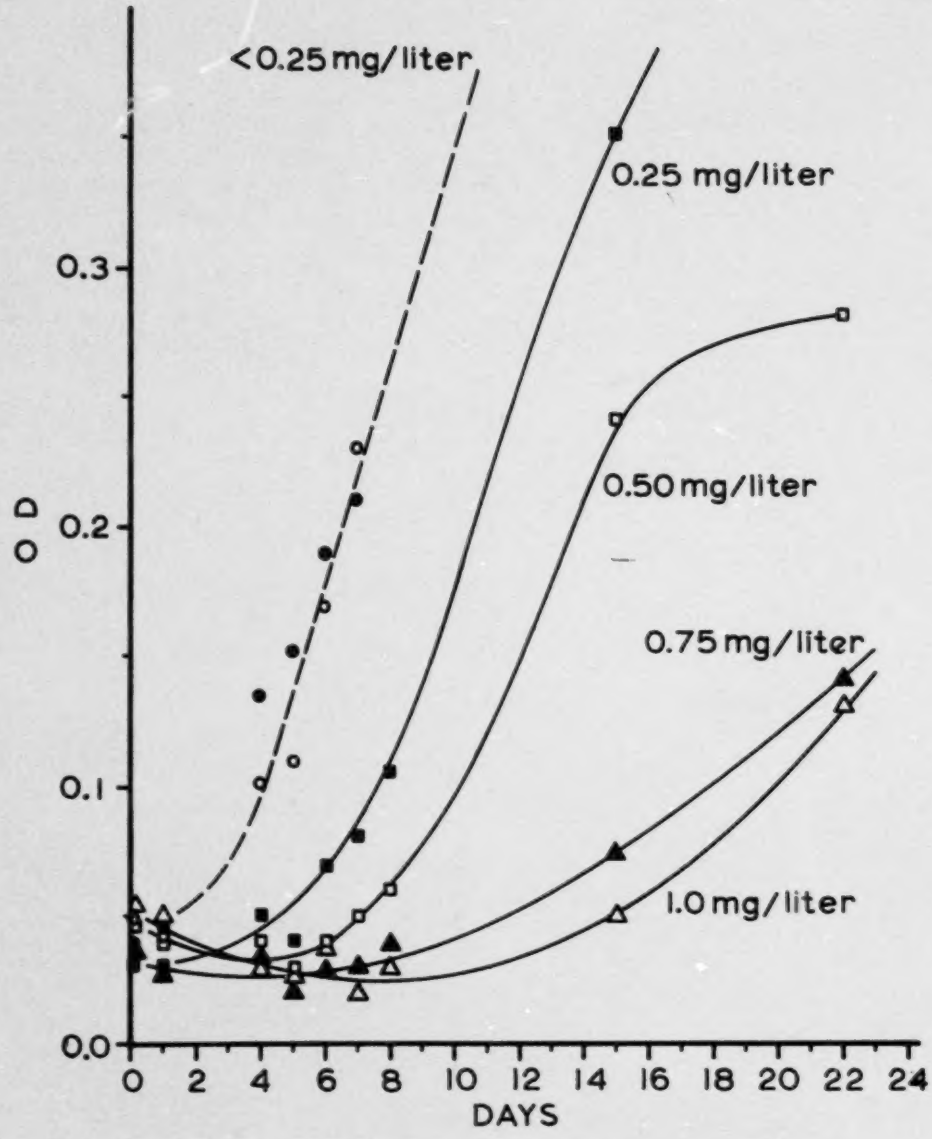


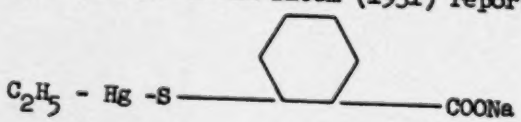
Fig. 8. The effect of mercuric chloride (as mg Hg per liter) on the growth of Scenedesmus (● = control; O = 0.1 mg).

contained numerous healthy-looking cells after 14 months and continued to show a gradual increase in density (Figure 6).

Although mercuric chloride proved to be an excellent bacteriostatic and algistatic agent, phytoplankton preserved with this substance developed an objectionable granular appearance which interfered with counting and identification, and was very different from the appearance of algae in Merthiolate-preserved samples.

Water Pollution Surveillance System Preservative Formula

The search for more effective organic mercurials launched by the pharmaceutical industry following World War I led to the synthesis of a number of compounds which possessed vastly improved germicidal properties. One of these, Merthiolate (sodium ethyl-mercuri-thiosalicylic acid; 50% mercury), has been a household disinfectant for more than a generation. Soon after its synthesis (Kharasch, Shonle, and Waldo, 1927; Waldo, 1931), Merthiolate was found to be bactericidal at very low concentrations. Buchsbaum and Bloom (1931) reported that a



concentration of 7 mg Merthiolate per liter was toxic to Micrococcus pyrogenes. Birkhaug (1933) rated its bactericidal strength as greater than that of mercuric chloride, but less than that of phenyl mercuric nitrate.

Despite its great effectiveness against pathogenic bacteria, Merthiolate has a relatively low human toxicity. Powell and Jamieson (1931) reported that an adult human could tolerate an intravenous injection of 250 mg of Merthiolate without developing symptoms of mercury poisoning. Rats tolerate 45 mg of Merthiolate per Kg of body weight, which would be comparable to a body burden of 3 grams for a 150 pound human. The lethal dose of mercuric chloride, in contrast, is only 70 to 100 mg (Thienes and Haley, 1964). Merthiolate is highly soluble in water (1 gm per ml), and does not coagulate proteins. These properties prompted Tatum et al. (1939) to recommend its use (at 100 mg per liter) to preserve human blood fractions. Merthiolate is currently marketed under the trade name "Thimerosol" by Eli Lily and Company, Indianapolis, Indiana,* for the preservation of human blood plasma and serum. It possesses many of the properties of the ideal preservative.

The preservative formula used routinely in Water Pollution Surveillance System plankton samples (Williams, 1962) contains Merthiolate, sodium borate (borax), and Lugol's solution. The Merthiolate and borate are used in the same proportion (1:1.5) as recommended by Eli Lily for blood preservation. The Lugol's solution is added to stain the starch in the green algae to aid in their identification during Sedgwick-Rafter counting.

*Mention of brand names or commercial sources does not constitute endorsement by the Federal Water Pollution Control Administration.

The solution is prepared by dissolving the following reagents in one liter of water:

a. 1.0 gram of Merthiolate

b. 1.5 grams of sodium borate (borax)

c. 1.0 ml of Lugol's solution (a saturated aqueous, iodine-KI solution prepared by dissolving 60 grams of KI and 40 grams of iodine in one liter of distilled water)

Plankton sample bottles shipped from our laboratory contain sufficient volume of this preservative solution to provide 36 mg of Merthiolate, 54 mg of sodium borate, and 1.3 mg of iodine per liter of water sample when the bottle is filled (36 ml of preservative solution is added per liter of bottle capacity). The cost of materials is approximately $0.01 per liter of sample. This formula effects excellent color retention, and causes no morphological distortions or cell shrinkage. Grab samples may be stored on the shelf for a year without deteriorating (Jackson and Bender, 1964; Weber, 1966).

Bacteriological Tests

Four grab samples, collected from the Little Miami River over a period of one year, were plated for initial bacteria counts, and then subdivided into control (raw) and treated aliquots, and allowed to stand on the shelf for 10 days before replating. After 10 days, the total bacteria counts in the preserved aliquots (containing 36 mg

Merthiolate, 54 mg sodium borate, and 1.3 mg iodine per liter) were similar to those in the raw samples (Table 2), indicating that the preservative had no effect on the bacterial flora.

Table 2. Total bacteria counts in raw and preserved Little Miami River water (organisms/ml)

Sample	Initial Count (Raw Sample)	Count after 10 days	
		Control (Raw)	Preserved
A	2×10^5	2×10^6	3×10^6
B	6×10^3	6×10^6	4×10^6
C	5×10^4	3×10^5	3×10^6
D	1×10^4	2×10^6	2×10^5

Despite the apparent lack of bacterial control at the Merthiolate concentration normally used in Water Pollution Surveillance System samples, no algal deterioration has been observed in stored samples.

Algal Bioassays

Cultures of Cyclotella and Scenedesmus were treated with stock preservative formula which was diluted to provide the following concentrations of mercury (as Merthiolate): 0.01, 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 mg per liter. The growth of Cyclotella was not affected at 0.01 and 0.1 mg Hg per liter, but was delayed two weeks by 0.5 mg, and for more than one year at 1.0, 2.0, 5.0, and 10.0 mg Hg per liter (Figure 9). The growth of Scenedesmus was also unaffected at 0.01,

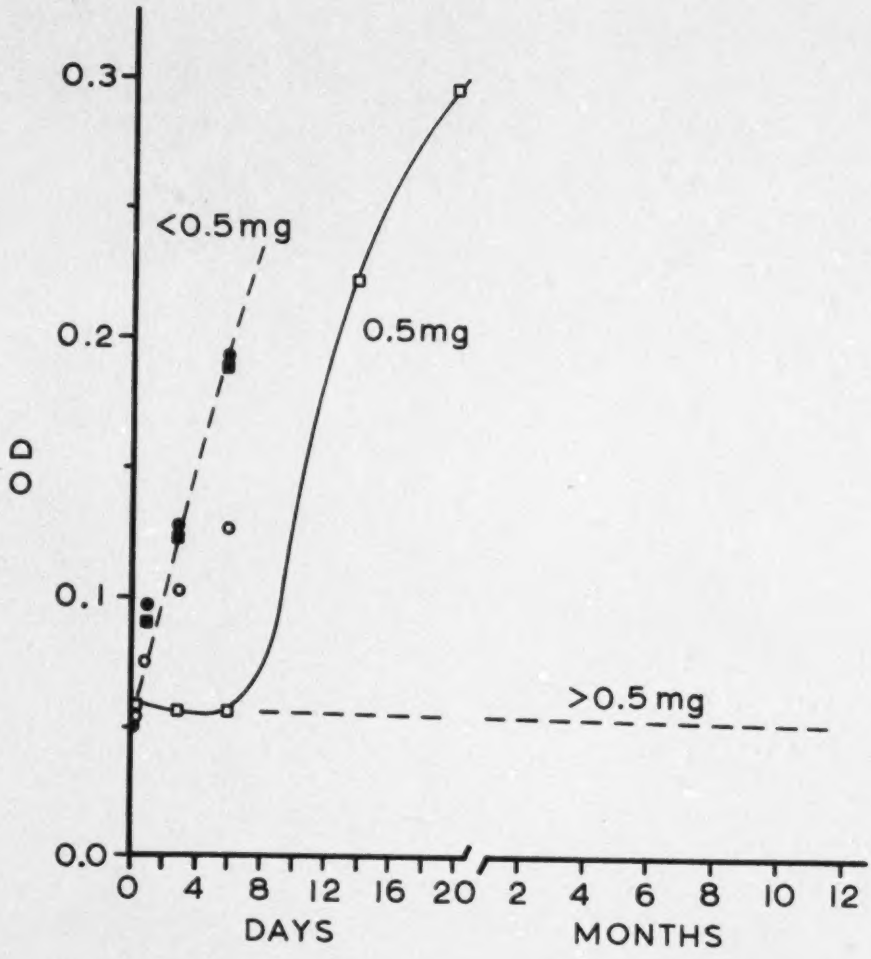


Fig. 9. The effect of the "complete" Merthiolate preservative (as mg Hg per liter) on the growth of Cyclotella (● = control; 0 = 0.01 mg; values >0.5 mg include - 1.0, 2.0, 5.0, and 10.0 mg).

and 0.1 mg Hg per liter, but was delayed two weeks by 0.5 mg, six weeks by 1.0 mg, six months by 2.0 mg, and more than one year by 5.0 and 10.0 mg mercury per liter (Figure 10).

The Merthiolate preservative formula proved to be much more effective against the algae than the bacteria. It was evident that the concentration of Merthiolate routinely used in WPSS samples provides a wide margin of safety against algal growth.

Shelf Life

Shipments of Merthiolate received from Eli Lily and Company were accompanied by directions for use which implied that aqueous solutions of the product rapidly lose their potency and should be discarded after six weeks of shelf storage. Powell and Jamieson (1938) reported that the potency of refrigerated Merthiolate solutions was unchanged after three years, but declined measurably in ten years. They made no mention, however, of solutions stored at room temperature.

The algistatic properties of stock solutions of the complete Merthiolate preservative which had been stored in the refrigerator and on the shelf for 36 months in our laboratory, were compared with those of freshly prepared preservative by treating Scenedesmus with 1/10, 1/2, and full strength solutions of each. Growth resumed after two weeks in the culture preserved with 1/10 strength shelf-stored preservative, after three weeks in the culture preserved with 1/10 refrigerated preservative, and after six weeks in the culture

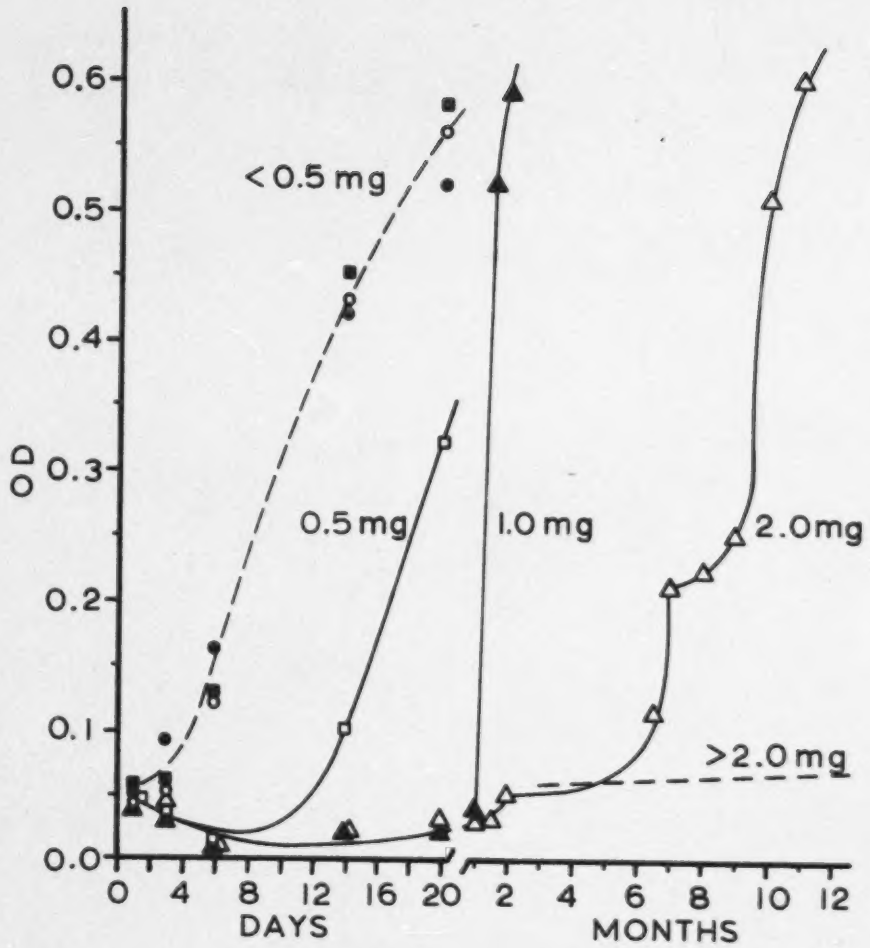


Fig. 10. The effect of the "complete" Merthiolate formula (as mg Hg per liter) on the growth of Scenedesmus (● = control; 0 = 0.01 mg; ■ = 0.1 mg; values greater than 2.0 mg include 5.0, and 10.0 mg).

preserved with 1/2 strength shelf-stored preservative. All other cultures were still in stasis at the end of four months. These observations indicated that some deterioration occurred in both the refrigerated and shelf-stored preservative. Used at full strength, however, stasis was effected for at least four months, and since the analysis of our samples is rarely delayed for more than this length of time, the deterioration of the preservative would not affect its usefulness.

Components of the Merthiolate-Sodium Borate-Iodine Formula

Merthiolate, borax, and Lugol's solution were tested separately and in combination to determine the algistatic properties of each, and to detect possible synergistic effects.

SODIUM BORATE

No bacteriological tests were made with sodium borate.

Algal Bioassays

Cultures of Cyclotella and Scenedesmus were treated with sodium borate at the following concentrations: 1, 2, 4, 10, 20, 40, and 100 mg per liter. The growth rates of the experimental cultures did not differ significantly from those of the controls.

Eli Lily and Company recommends the addition of borax to solutions of Merthiolate to maintain an alkaline condition, because the free acid of Merthiolate is much less soluble than the sodium salt. Other than a slight buffering effect, therefore, this substance probably has no effect on the preservation of the samples.

LUGOL'S SOLUTION

The concentration of iodine (1.3 mg per liter) routinely employed in our samples was considered to be too low to have a measurable affect on the bacteria. No bacteriological tests, therefore, were conducted.

Algal Bioassays

Cultures of Cyclotella and Scenedesmus were treated with Lugol's solution to provide the following concentrations of iodine: 1, 4, 10, 20, 40, and 100 mg per liter. The growth of Cyclotella cultures was not retarded at iodine concentrations of 1, 4, 10, and 20 mg per liter (Figure 11). Growth was delayed three months, however, at 40 and 100 mg iodine per liter. The growth of Scenedesmus was briefly delayed at 1 mg iodine per liter. The delay in growth lengthened with increasing iodine concentration, reaching three weeks at 40 mg per liter (Figure 12). Growth had not resumed at the end of five weeks in cultures treated with 100 mg iodine per liter.

These data indicated that at the concentration normally used in our samples, the iodine alone probably contributes little to the stabilization of the plankton populations. Evidence of a synergistic effect with Merthiolate was obtained, however, and is discussed later in this report.

MERTHIOLATE USED ALONE

In view of the many published reports regarding the excellent bactericidal properties of Merthiolate, it was expected that sample

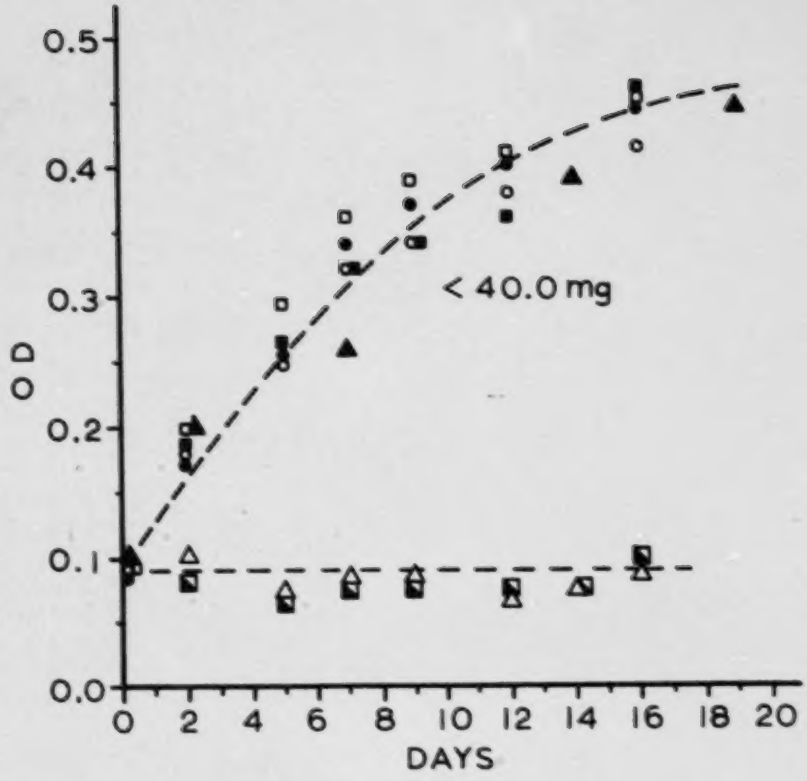


Fig. 11. The effect of Lugol's Solution (as mg I_2 per liter) on the growth of Cyclotella (● = control; 0 = 1.0 mg; ■ = 4.0 mg; □ = 10.0 mg; ▲ = 20.0 mg; △ = 40.0 mg; ◪ = 100.0 mg).

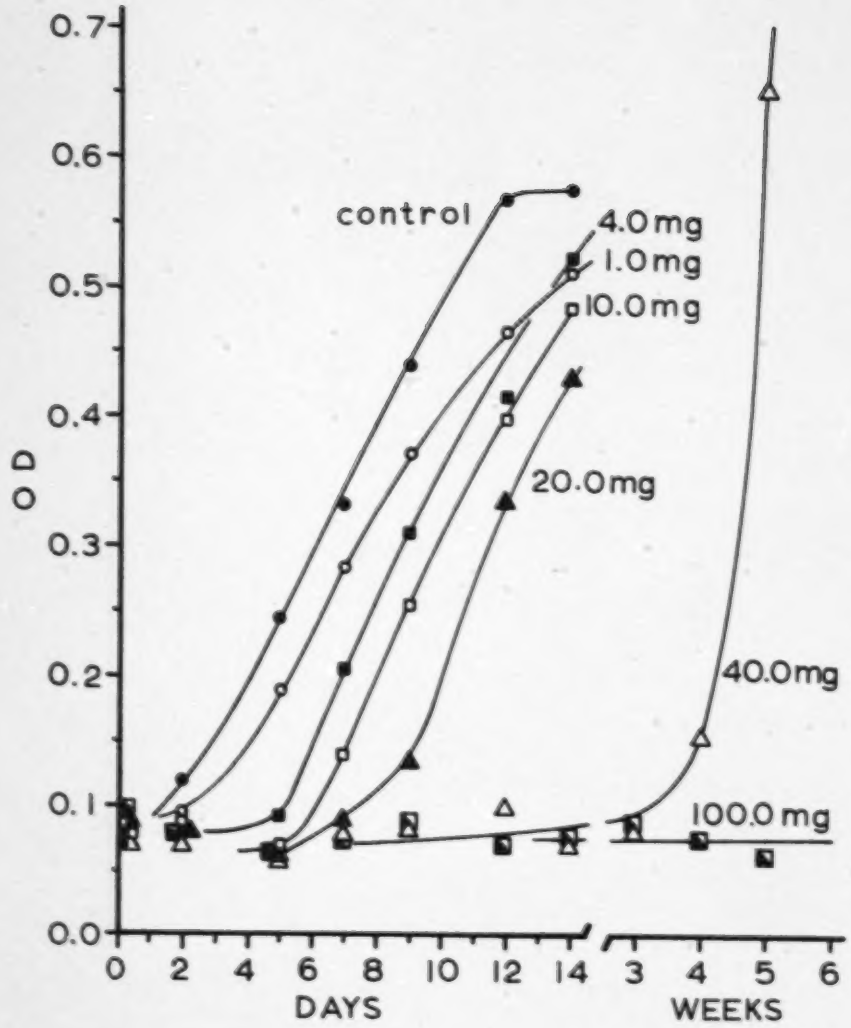


Fig. 12. The effect of Lugol's Solution (as mg I_2 per liter) on the growth of Scenedesmus.

sterility would be achieved at a low concentration. As mentioned earlier, however, the total bacteria counts in grab samples were not measurably reduced at a concentration of 36 mg Merthiolate per liter. Sterility of grab samples was not achieved even with 1 gm of Merthiolate per liter.

Algal Bioassays

Five series of cultures were treated with Merthiolate at concentrations ranging from 0.25 to 150 mg Hg (as Merthiolate) per liter. For the sake of brevity, however, only two series are discussed here in detail.

The growth of Cyclotella (Series A, Table 3) was affected only slightly at 0.5 and 1.0 mg Hg per liter, but was delayed seven days at 2.0 mg and 5.0 mg, and 32 days at 10.0 mg Hg per liter (Figure 13). Growth had not resumed at the end of seven months in cultures containing 20 mg and 40 mg of Hg per liter.

Scenedesmus was somewhat more sensitive to Merthiolate than was Cyclotella. The growth of Scenedesmus was delayed seven days at 0.5, 1.0, and 2.0 mg, 16 days at 5.0 mg, and 54 days at 10.0 mg of Hg per liter (Figure 14). No growth was observed in cultures containing more than 10 mg of Hg per liter.

A summary of the effects of Merthiolate on algae is presented in Table 3. Differences in the growth responses of the algae were most likely due to differences in water quality (pH, nutrient levels, etc.) and algal sensitivity.

Table 3. The delay in algal growth (d = days; M = months) at different concentrations of Merthiolate-mercury

Culture Series	Culture Medium	Mg mercury (as Merthiolate) per liter									
		0.25	0.5	1	2	4-5	10	20	40	80	150
A	Chu #10	a	1d	1d	7d	7d	33d	>7M	<7M[2]	a	a
B	Chu #10	a	7d	7d	7d	16d	54d	>7M	>7M	a	a
C	Ohio River Water	4d	6d	8d	13d	20d	32d	>5M	>5M	a	a
D	Little Miami River Water	4d	6d	8d	12d	20d	46d	>5M	>5M	a	a
E	Ohio River Water	a	a	14d	28d	>4M	>4M	>4M	>4M	>4M	>4M

1. a - no culture at that concentration

2. all cultures with "more than" time values were still under observation when this report was written

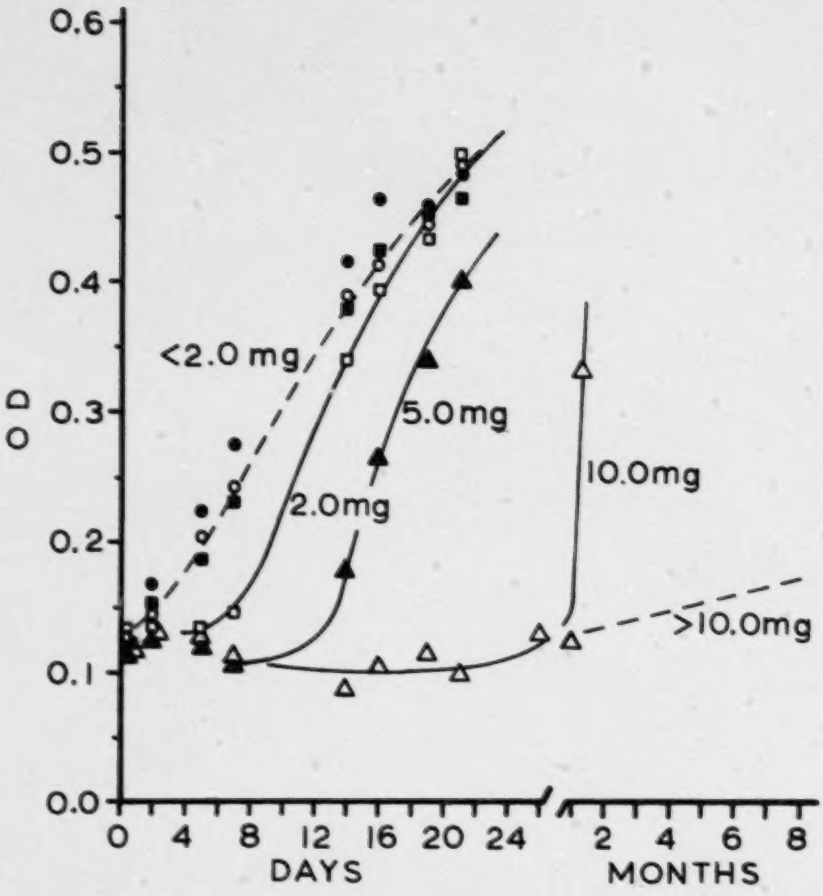


Fig. 13. The effect of Merthiolate alone (as mg Hg per liter) on the growth of Cyclotella (● = control; ○ = 0.5 mg; ■ = 1.0 mg; values greater than 10.0 mg include 20.0 mg and 40.0 mg).

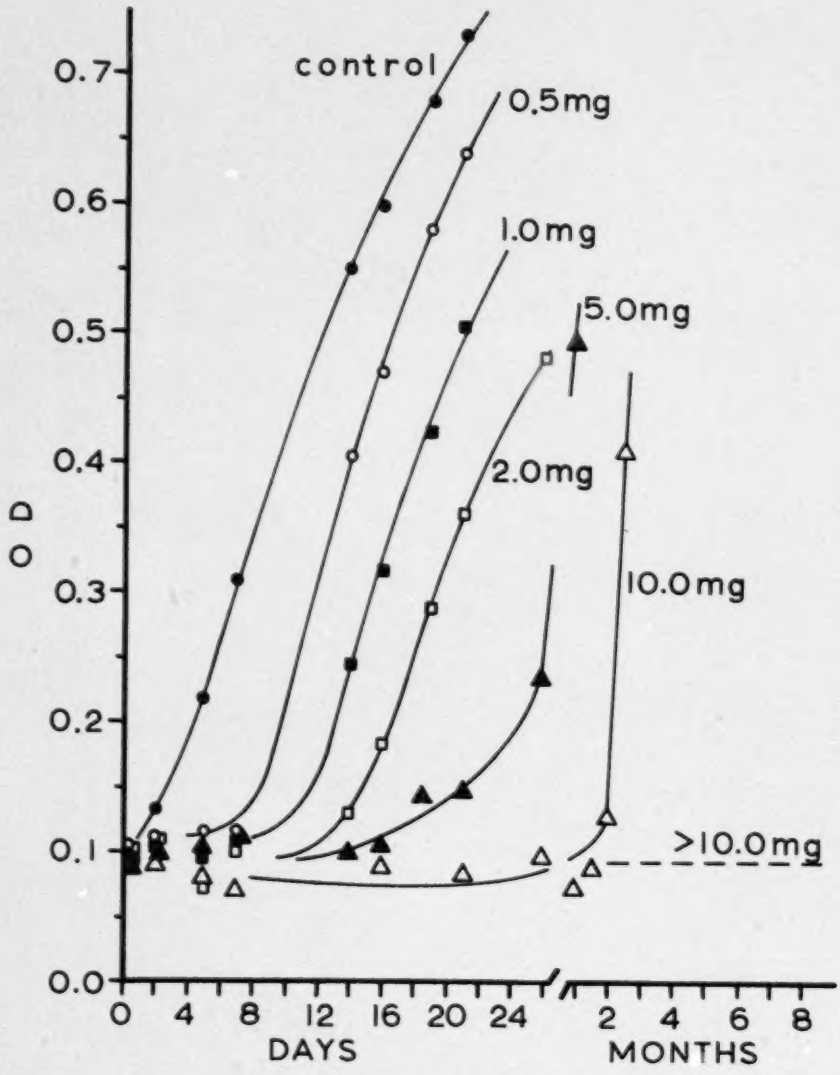


Fig. 14. The effect of Merthiolate alone (as mg Hg per liter) on the growth of Scenedesmus (values greater than 10.0 mg include 20.0 mg and 40.0 mg).

The data presented here show that Merthiolate was much more effective against the algae than the bacteria. Merthiolate was not as effective against the algae when used alone as it was when used in combination with sodium borate and Lugol's solution. This result suggested a synergistic reaction. This possibility was explored by testing the algistatic properties of Merthiolate in combination with borax or iodine.

MERTHIOLATE WITH SODIUM BORATE

Scenedesmus cultures were treated with 1 mg Merthiolate per liter and with sodium borate at the following concentrations: 1, 2, 4, 10, 20, and 40 mg per liter. Growth was delayed for 7 days in all cultures due to the effect of the Merthiolate, but there was no evidence of an interaction between the Merthiolate and the borate (Figure 15). The cultures containing 2, 4, and 10 mg sodium borate per liter appeared somewhat more dense than the others when the experiment was terminated (19 days). It is possible that the growth of the algae was stimulated at those concentrations of borate, but this effect was not studied further.

MERTHIOLATE WITH LUGOL'S SOLUTION

Two series of Scenedesmus cultures were prepared with Merthiolate and Lugol's soltuion - one in Chu Medium #10, and one in filter-sterilized Ohio River water.

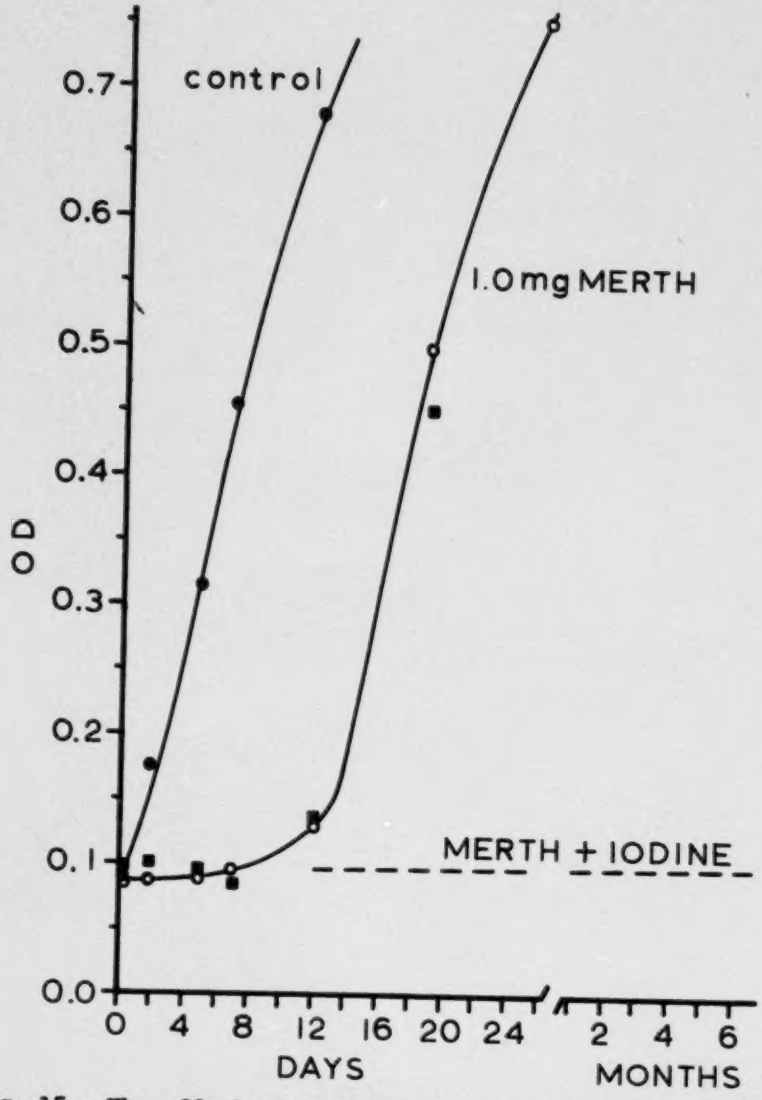


Fig. 15. The effect of Merthiolate with borax, and Merthiolate with iodine on the growth of Scenedesmus (● = control; ○ = 1.0 mg Merthiolate per liter; ■ = 1.0 mg per liter Merthiolate with 40 mg per liter borax; "Merthiolate + iodine" values involve 1 mg Merthiolate in combination with the following levels of iodine - 1.0, 2.0, 5.0, 10.0, 20.0 and 40.0 mg per liter).

The first series contained 1 mg Merthiolate per liter, with the addition of iodine (I_2 as $KI \cdot I_2$) in the following concentrations: 1, 2, 5, 10, 20, and 40 mg per liter. Growth was delayed one week in the control culture containing only Merthiolate, but had not resumed within six months in any of the samples treated with iodine (Figure 15).

In order to define more precisely the level of iodine needed to effect stasis, the second series was prepared which contained 2 mg Merthiolate per liter, and iodine in the following concentrations: 0.01, 0.05, 0.1, 0.5, and 1.0 mg per liter. Growth was delayed three weeks in the control culture containing only Merthiolate, and in the cultures containing Merthiolate with 1 mg or less of iodine per liter. Growth had not resumed in four months in the cultures containing Merthiolate with 2 mg or more of iodine per liter.

The ability of low concentrations of Merthiolate to effect long periods of algal stasis in the presence of 1-2 mg per liter of iodine may result from: (a) a synergistic effect, and/or (b) a direct chemical interaction between the Merthiolate and iodine, which yields a product that is more algicidal than either reactant. The likelihood of a chemical interaction is suggested by the reduction of the iodine color of Lugol's solution when Merthiolate is added. This reaction deserves further study.

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